SOME PHYSICAL AND CHEMICAL FACTORS AFFECTING ASEXUAL REPRODUCTION OF THREE *PYTHIUM* SPP.

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ABSTRACT *Pythium aphanidermatum* produced zoospores most abundantly at 35° C, whereas the optima for *P. oligandrum* and *Pythium* "group F" were 15 and 25° C respectively. The optimum temperature of mycelial growth was 30° C for all species tested. Optimum pH for mycelial growth and zoospore production was 7 for tested fungi. Moderate osmotic potentials (-0.13 -0.27 MPa) appeared to favour either mycelial growth or zoospore production. Low salinity (0.1 0.5 % NaCl) was satisfactory for mycelial growth and zoospore production.

KEY WORDS mycelial growth, osmotic potential, pH, salinity, temperature, zoospores, *Pythium aphanidermatum*, *Pythium oligandrum*, *Pythium* "group F"

RESUME La température optimale pour la production de zoospores est de 35° C et 25° C respectivement pour le *Pythium aphanidermatum* et le *Pythium* "groupe F". La température optimale de croissance est de 30° C pour toutes les espèces de *Pythium* testées. Un pH de 7, un potentiel osmotique modéré (-0.13 -0.27 MPa) et une salinité faible (0.1 0.5 % NaCl) favorisent la croissance et la production de zoospores.

MOTS-CLEFS croissance mycélienne, potentiel osmotique, pH, salinité, température, zoospores, *Pythium aphanidermatum*, *Pythium oligandrum*, *Pythium* "groupe F"

INTRODUCTION

Asexual reproduction in most species of *Pythium* takes place by means of zoosporangia which produce zoospores under wet conditions. However zoosporangia can germinate directly by germ tubes under certain conditions and especially when water is lacking. A group of pythia were found to fail to produce zoospores even in the presence of water (Plaats-Niterink, 1981). Many species of *Pythium* occurring in water basins and in water streams spread by water currents from place to place. Water quality and environmental factors proved to be important for zoospore formation by *Pythium* spp. (Abdelzaher, 1994, Abdelzaher *et al.*, 1994).

Abdelzaher *et al.*, (1994) studied zoospore formation by three pythia namely *P. fluminum* Park var. *fluminum*, *P. marsipium* Drechsler and *Pythium* "group F". He repor-

ted temperature, pH value, and osmotic potential affect zoospore formation of these three species.

P aphanidermatum (Edson) Fitzp is a widespread fungal pathogen occurring mainly in warm regions (Middeleton, 1943, Waterhouse, 1967, 1968, Plaats Niterink, 1981 and Dick, 1990). Data concerning zoospore formation by this fungus under various environmental conditions could be of importance for our knowledge of its ecological distribution. *P oligandrum* is an aggressive mycoparasite of several fungi (Deacon & Henry, 1978 and Plaats Niterink, 1981) and is thus used in the biological control of some fungal diseases (Plaats Niterink, 1981). This role verifies its use in this study in order to understand factors affecting its establishment in soils. On the other hand *Pythium* "group F", a typical aquatic fungus, relies on zoospores for dissemination and factors affecting their formation is no doubt important.

The aim of this work was to examine the effect of temperature, hydrogen ion concentration, osmotic potential and salinity on mycelial growth and zoospore production by *P aphanidermatum*, *P oligandrum* and *Pythium* "group F".

MATERIALS AND METHODS

P aphanidermatum, *P oligandrum* were isolated from an agricultural field located close to El-Ibrahimia canal near El Minia City, Egypt. *Pythium* "group F" was isolated from the water of this canal at the same site. Baiting techniques using cucumber seeds, filter paper discs and *Paspalum* leaf blades were used for isolation (Abdelzaher, 1994, 1995, Abdelzaher *et al*. 1994, Pittis & Colhoun, 1984). Fungi were inoculated in Petri dishes containing 3% water agar until the developing colonies reached about 4 cm diam. Then autoclaved pieces (3x14 mm) of *Zea mays* leaf blades were laid over each colony and in contact with the actively growing margin and incubated at 25° C. After 24 h incubation, colonized *Zea mays* leaf blades were transferred to Petri-dishes containing 10 ml of sterilized distilled water to determine the effect of temperature on zoospore production.

To study the effect of Hydrogen-ion concentration, colonized *Zea mays* leaf blades were transferred to Petri-dishes containing 10 ml of 0.05 M acetate or Tris-HCl buffers previously adjusted to pH values from 4 to 9. Another experiment using solutions of different pH values were prepared using HCl and NaOH. All solutions and buffers were sterilized by filtration.

To study the effect of osmotic potential, the method of Abdelzaher *et al*. (1994) was used. Colonized *Zea mays* leaf blades were transferred to Petri-dishes containing 10 ml of sterilized mannitol solution dissolved in distilled water, to give different osmotic potentials. Distilled water was used as a control (Zero MPa osmotic potential).

To study the effect of salinity, colonized *Zea mays* leaf blades were transferred to Petri-dishes containing 10 ml portions of autoclaved NaCl solutions with different concentrations.

Cultures were observed microscopically along the *Zea mays* leaf margin through the lid of the Petri-dish at different intervals. *P aphanidermatum* has sporangia consisting of terminal complexes of swollen hyphal branches of varying lengths and which produce vesicles at the top of evacuation tubes. *P oligandrum* has spherical sporangia with complex subglobose elements and these produce vesicles at the top of evacuation tubes. But *Pythium* "group F" has filamentous, non-inflated zoosporangia which produce vesicles at the top of long evacuation tubes. Each active vesicle contains zoospores ready to be

discharged after a specific time. An active vesicle can be determined by its zoospore differentiation just before discharge. To record numbers of such active vesicles along the leaf margin, the methods of Saleem & Dick (1990) and Abdelzaher *et al.* (1994) were followed. A mean value was calculated for each treatment and expressed as number of vesicles per mm. The whole experiment was repeated twice with three replicates.

To examine the effect of temperature on mycelial growth, 15 ml of cornmeal agar (CMA) were placed in 9 ml Petri-dishes, inoculated with the test fungus and incubated at different temperatures. To study the effect of hydrogen-ion concentration, 2 % agar was adjusted to different pH values using the buffer systems described previously. Mycelial discs (5.0 mm diam) of the test fungi were cut from the periphery of 3 d old cultures, inoculated in the center of each assay plate and then incubated at 28 ± 1° C.

The effect of osmotic potential and salinity were studied using 2 % agar prepared according to the experimental conditions required as described previously for zoospore formation.

RESULTS

Figure 1 shows zoospore production was generally high after 24 h incubation and it then gradually decreased with time. *P. aphanidermatum* started to produce zoospores after 24 h incubation at 15, 20, 25 or 30° C. At lower temperatures (5, 10 and 15° C), zoospore production continued for several days, till the end of the experimental period whereas at high temperatures production declined drastically. *P. oligandrum* began to produce zoospores after 24 h at 25, 30 and 35° C but at lower values (10, 15, 20° C) it produced zoospores only after 48 h. Low temperatures supported a longer time of zoospore production by *P. oligandrum*. *Pythium* "group F" started zoospore production after 24 h between 5 and 35° C, but it failed to produce zoospores at 40° C. It follows that low temperatures supported a longer time of zoospore production than higher ones.

Figure 2 shows that production of zoospores was inhibited at pH 4 in the case of *P. aphanidermatum* and *P. oligandrum*. All pythia tested could however produce zoospores within the pH range 5-9. Exception is *P. aphanidermatum* which could not produce zoospores at the higher value. Optimum pH for zoospore production was 7 for the three fungi used. In solutions prepared, using HCl or NaOH for pH adjustment, all pythia tested produced zoospores from pH 5.5-9.5 but their formation was inhibited at pH 10.5 (data not included).

Figure 3 represents the effect of osmotic potential on zoospore production for the three species. Distilled water (zero osmotic potential) was favourable for good production by tested fungi. In distilled water containing mannitol to adjust osmotic potential between -0.13 MPa and -1.00 MPa, also all species were able to produce zoospore between -0.13 to -0.47 MPa but not above this range, recorded optimum was at osmotic potential at -0.27 MPa.

Figure 4 shows that production of zoospores by *P. aphanidermatum* was inhibited by 1 % NaCl. *P. oligandrum* and *Pythium* "group F" could produce zoospores at 2 % NaCl while at higher concentrations this process was inhibited. The optimum concentration was 0.2 % NaCl.

Tables 1, 2, 3 and 4 respectively represent minimum, optimum and maximum values of temperature, hydrogen-ion concentration, osmotic potential and salinity for mycelial growth of each of the three fungi. The minimal temperatures of *P. aphanidermatum*, *P. oligandrum* and *Pythium* "group F" were 15, 10 and 10° C, respectively while their

Pythium spp.	Daily growth rate [Diameter increase 24 h (mm)]							
	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
P aphanidermatum	0	0	4*	12	29	34	26	16
P. oligandrum	0	3	8	13	16	24	6	3
Pythium "group F"	0	3	5	8	10	13	11	0

Table 1 Effect of temperature on mycelial growth of three *Pythium* spp. in CMA medium
Tableau 1 Effet de la température sur la croissance mycélienne de trois *Pythium* sp. sur milieu CMA

* All results are the mean of three replicates with standard errors not exceeding 0.5

Pythium spp.	Daily growth rate [Diameter increase / 24 h (mm)]					
	pH4	pH5	pH6	pH7	pH8	pH9
P aphanidermatum	0	0	18*	28	27	20
P. oligandrum	0	0	8	24	15	9
Pythium "group F"	0	0	6	14	4	2

Table 2 Effect of pH value on mycelial growth of three *Pythium* spp. cultivated in CMA medium at 28° C
Tableau 2 Effet du pH sur la croissance mycélienne de trois *Pythium* sp. sur milieu CMA à 28° C

* All results are the mean of three replicates with standard errors not exceeding 0.5.

Pythium spp.	Daily growth rate [Diameter increase / 24 h (mm)]				
	Dist. H2O	-0.13 MPa	-0.27 MPa	-0.47 MPa	-0.1 MPa
P. aphanidermatum	33*	31	27	[illegible]	0
P. oligandrum	22	20	16	4	0
Pythium "group F"	12	12	11	2	0

Table 3 Effect of different osmotic potentials on mycelial growth of three *Pythium* spp. cultivated in CMA medium at 28° C
Tableau 3 Effet du potentiel osmotique sur la croissance mycélienne de trois *Pythium* sp. sur milieu CMA à 28° C

* All results are the mean of three replicates with standard errors not exceeding 0.5

Pythium spp.	Daily growth rate [Diameter increase / 24 h (mm)]								
	Control	0.1%	0.2%	0.3%	0.4%	0.5%	1.0%	2.0%	3.0%
P. aphanidermatum	34	22	22	24	29	30	27	13	10
P. oligandrum	21	23	22	22	23	22	13	4	0
Pythium "group F"	13	16	11	15	14	13	9	7	4

Table 4. Effect of NaCl concentration on mycelial growth of three *Pythium* spp. cultivated in CMA medium at 28° C.
Tableau 4. Effet de la concentration en NaCl sur la croissance mycélienne de trois *Pythium* sp. sur milieu CMA à 28° C.

* All results are the mean of three replicates with standard errors not exceeding 0.5.

optimum was at 30° C. Maximum temperature was over 40° C for *P. aphanidermatum* and *P. oligandrum* but it is slightly lower (35-40° C) for *Pythium* "group F".

Experiments of the effect of pH values using buffers and 2 % agar on mycelial growth at 28° C, (Table 2) showed growth to occur within the range of 6.9 with a recorded overall optimum at pH 7. However growth was inhibited at lower values.

The effect of osmotic potential on mycelial growth at 28° C (Table 3) indicate similar responses in tested pythia. No growth occurred at -1.00 MPa, whereas at other tested osmotic potentials (-0.13–-0.27 MPa), good growth was observed at an optimum at -0.13 MPa.

The influence of NaCl concentration on mycelial growth at 28° C is shown in table 4. At a high concentration (3 %), *P. aphanidermatum* and *Pythium* "group F" grew poorly while *P. oligandrum* made no growth. At concentrations ranging from 0.1–0.5 % NaCl, good growth was obtained in all species, recorded optima were at 0.5, 0.4 and 0.1 % NaCl for *P. aphanidermatum*, *P. oligandrum* and *Pythium* "group F", respectively. Mycelial growth decreased at concentrations above 1 %.

DISCUSSION

One of the important roles of zoospores is to serve for the dissemination of fungi. In aquatic pythia this function is affected by water quality (Tomlinson, 1958 a, b). The present investigation clearly underline that water temperature, pH, osmotic potential and salinity are important parameters affecting zoospore formation and dissemination by *Pythium* spp.

Minimum temperatures for mycelial growth ranged between 10–15° C. *P. aphanidermatum* and *Pythium* "group F" produced zoospores at 5° C indicating optimum temperatures for zoospore formation is not necessarily coinciding with that of mycelial growth. At higher values, only *P. oligandrum* could produce a small amount of zoospores (at 40° C) for a short time, while *P. aphanidermatum* and *Pythium* "group F" could not produce zoospores at 40° C.

Acidic solutions (pH 4, 5) proved unsuitable for growth of all species. Such allowed the production of small amount of zoospores in only *Pythium* "group F". This result is in harmony with Suzuki (1961), Suzuki & Takashi (1966) and Abdelzaher (1994) statements that "zoospores of *Pythium* species were very rare in acidotrophic and polluted ponds and lakes". For this reason, acidic waters or soils do not support *Pythium* growth or its zoospore formation. Neutral pH was found to provide good condition for mycelial and zoospore formation by *Pythium* species.

Moderate osmotic potential appeared to favour zoospore production. This phenomenon is correlated with the influence of osmotic strength on the formation of the vesicles since at higher concentration levels, although fungi could produce vesicles, these failed to produce zoospores. Harvey (1952), in his study on the role of fungi in stream sanitation and processes of natural purification, concluded that no *Pythium* had been detected in heavily polluted waters and had seldom been found in partially polluted places. For the above reason, it is speculated that highly polluted soil and water with high osmotic potential act as a barrier against zoospore production by *Pythium* spp.

A concentration of 0.2 % NaCl was found suitable for zoospore production. Above this level a drastic decrease in this process was recorded. This phenomenon can be explained on the basis of the effect of salinity on vesicles formation. *Pythium* vesicle has a thin membrane sensitive to the surrounding medium. This permeable membrane needs a special NaCl concentration for complete differentiation. A level of 0.2 % NaCl matched the ideal concentration and appeared to favour zoospore production.

We can conclude that water quality influence mycelial and zoospore production by *Pythium* spp. Highly acidic, heavily polluted and saline waters do not support mycelial and zoospore formation by these fungi. However a generalization cannot be made and variation can occur throughout pythia.

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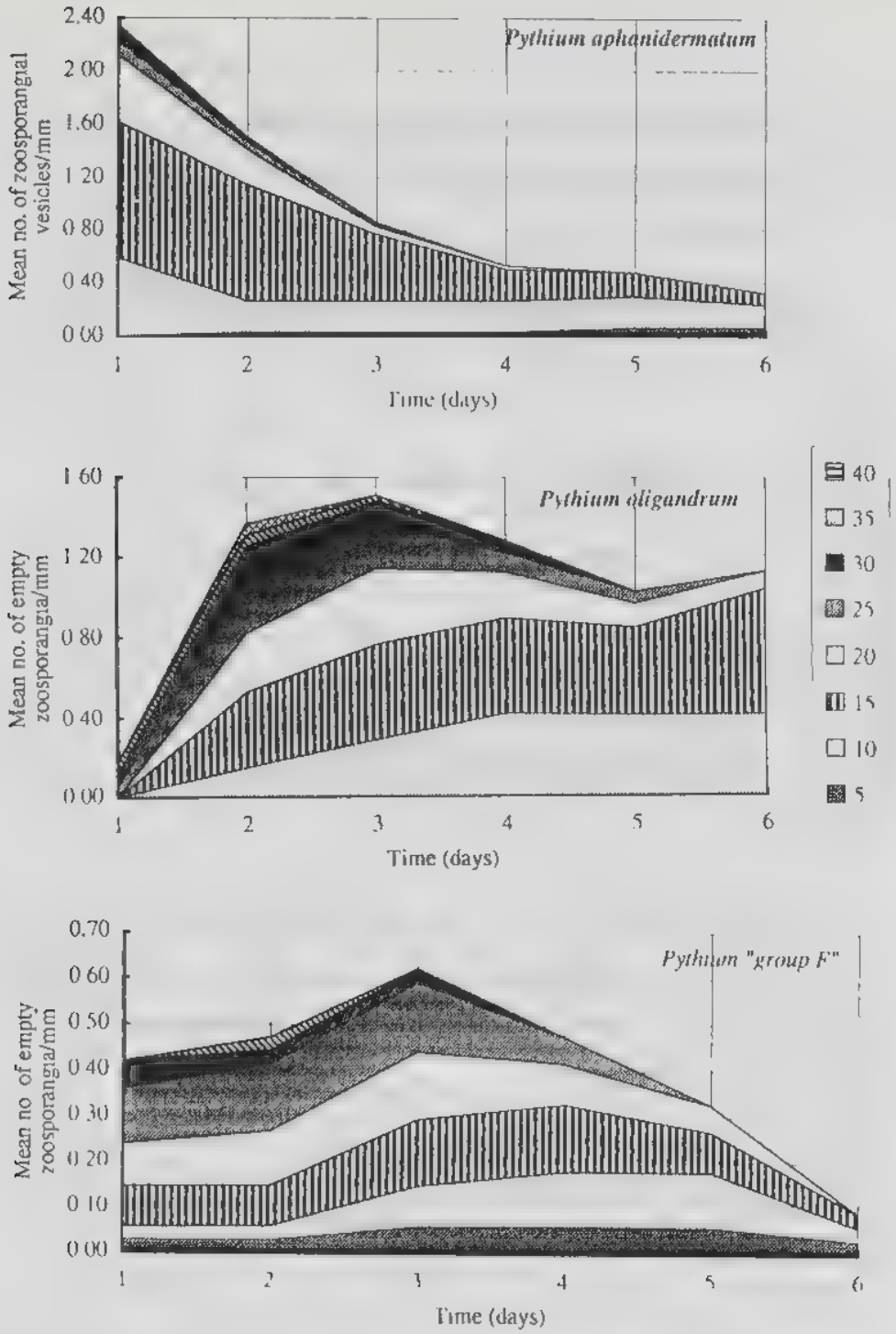

Fig. 1 - Effect of temperature on zoospore production by *Pythium* spp.
Fig. 1. — Effet de la température sur la production de zoospores par *Pythium* sp.

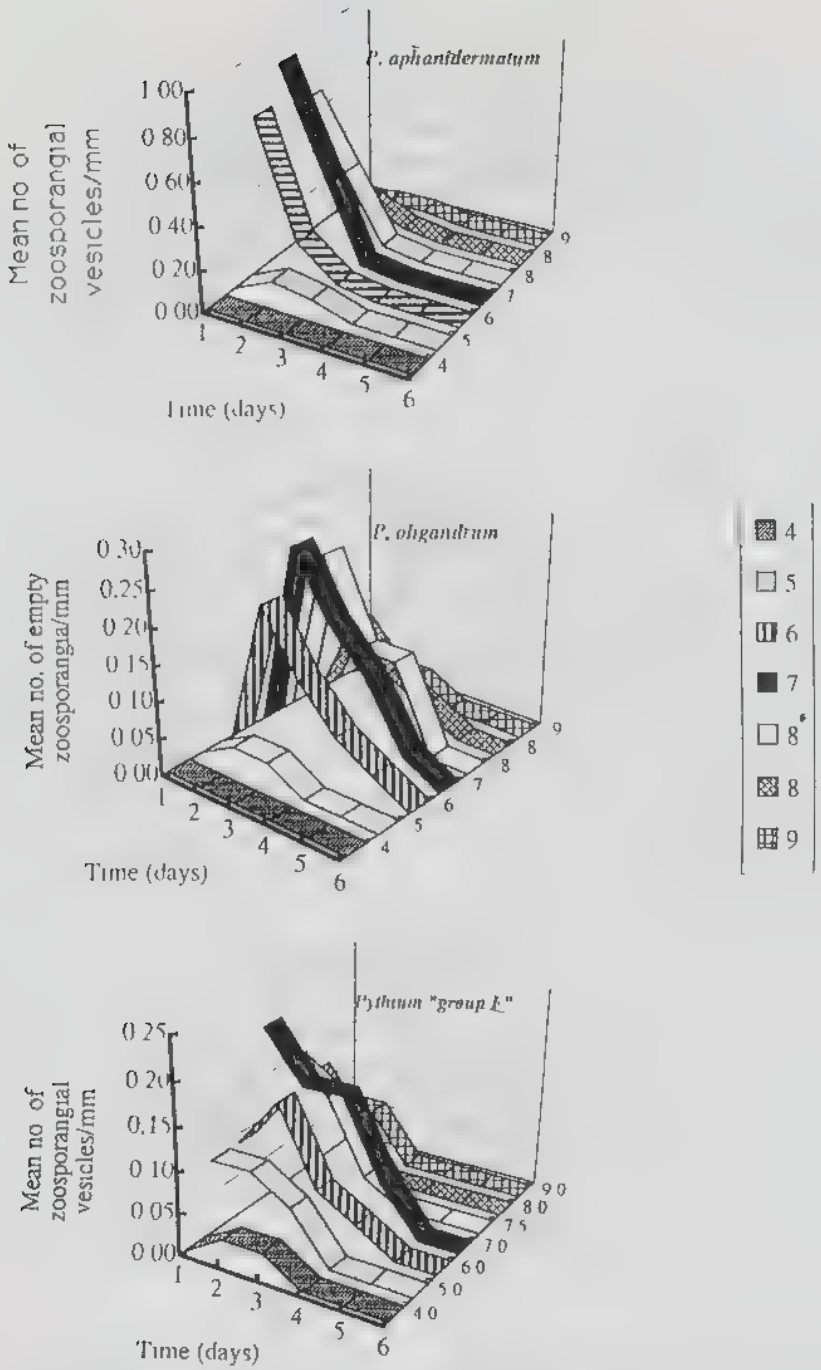

Fig 2 Effect of hydrogen ion concentration on zoospore production by *Pythium* spp at 28° C
Fig 2. Effet du pH sur la production de zoospores par *Pythium* sp

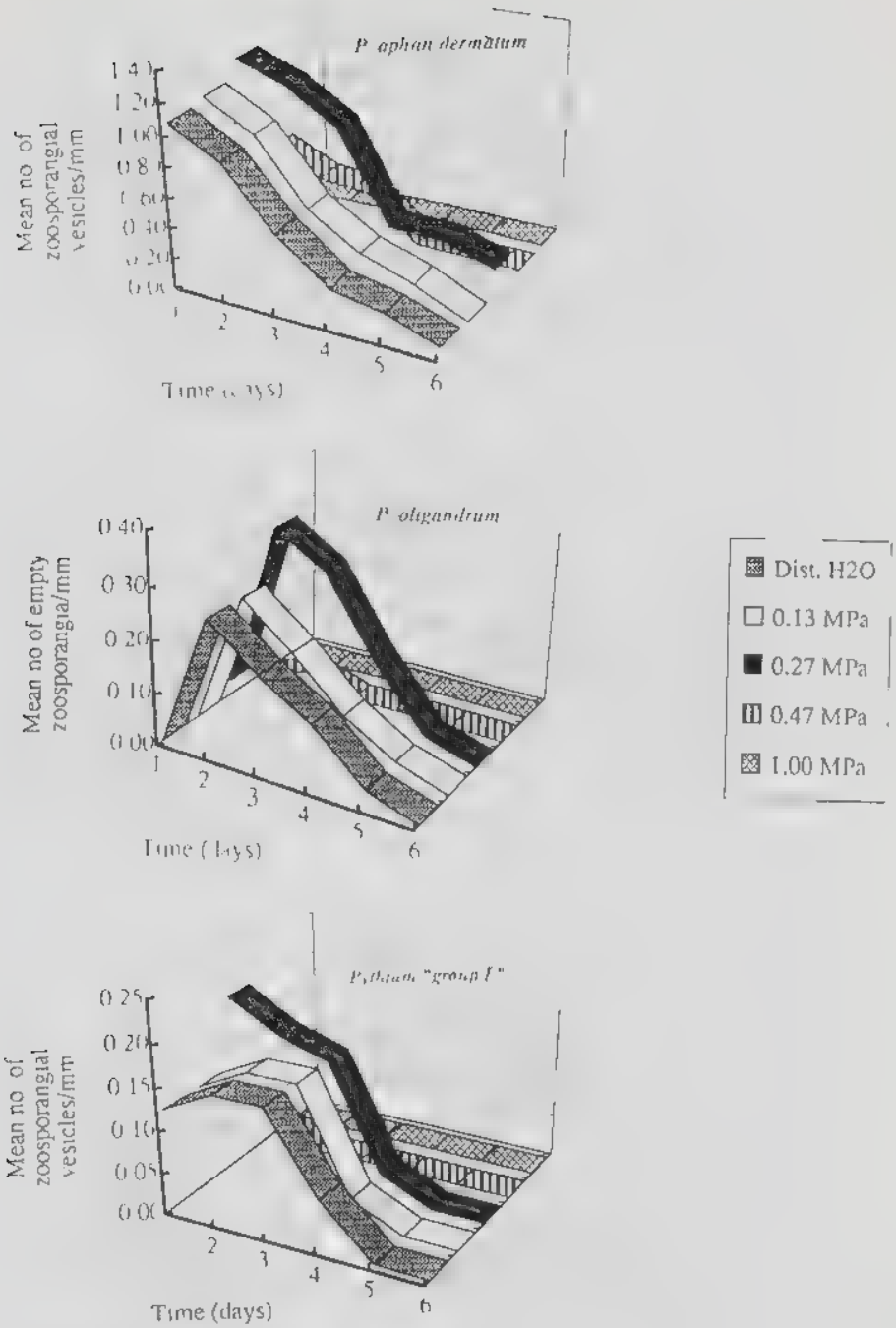

Fig 3 Effect of osmotic potential on zoospore production of three *Pythium* spp. at 28° C
Fig 3 Effet du potentiel osmotique sur la production de zoospores par trois *Pythium* sp. à 28° C

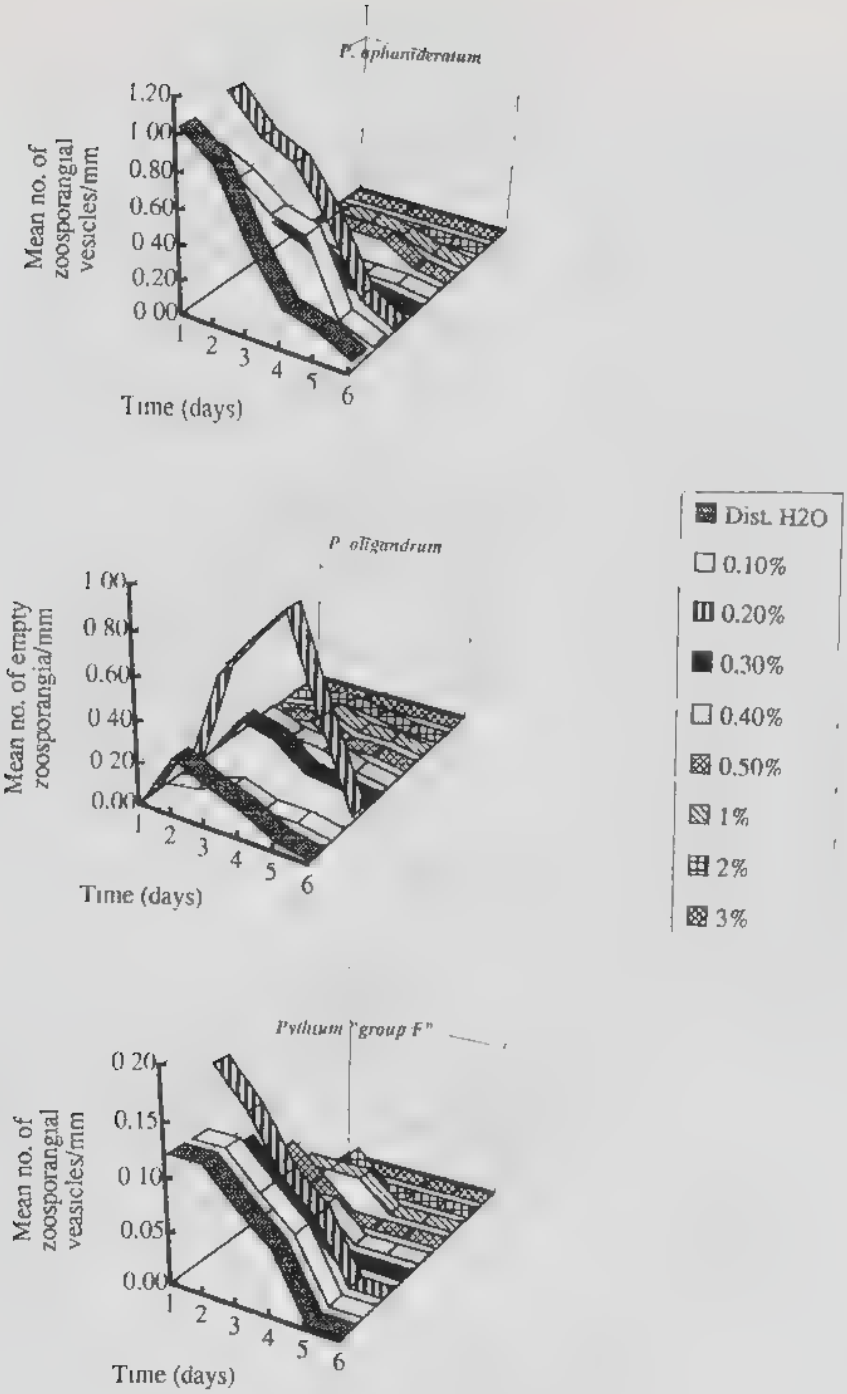

Fig. 4 Effect of salinity on zoospore production by *Pythium* spp. at 28° C
Fig. 4 Effet de la salinité sur la production de zoospores par *Pythium* sp. at 28° C